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File: USPT

Oct 12, 1999

DOCUMENT-IDENTIFIER: US 5965542 A

TITLE: Use of temperature to control the size of cationic liposome/plasmid DNA complexes

Detailed Description Text (60):

DODAC:DOPE liposomes (50:50 mol %) were prepared according the method of Hope et al, Biochem. Biophys. Acta 812:55-65 (1985). Lipids were dissolved in chloroform (20 mg/mL), radiolabeled at a specific activity of 1-2 $\mu\text{Ci}/50\text{ mg}$ with ^{14}C -CHE as a non-metabolizable and non-exchangeable liposomal marker (Scherphof et al., Biochem. Soc. Trans. 15:625-628 (1987)). For tracking radiolabeled lipid following injection, ^{14}C -DOPE was used as the liposomal marker. The lipids were dried to a thin film under a stream of nitrogen gas and vacuum dried at $>76\text{ cm Hg}$ for at least 4 hours. The films were hydrated in filter sterilized 300 mM lactose and passed 10 times at room temperature through an extruder (Lipex Biomembranes, Vancouver, B.C.) containing 3 stacked 80 nm polycarbonate membranes. The lipid concentration of the resulting liposome vesicles was calculated by multiplying the ratio of dpms from ^{14}C -CHE or ^{14}C -DOPE (Packard TR 1900 Scintillation Counter) before and after extrusion by the initial known lipid concentration. The size of the liposomes was measured by QELS using a Nicomp Submicron Particle Sizer (Model 270, Pacific Scientific, Santa Barbara, Calif.) operating at a wavelength of 632.8 nm. All liposomes had a mean diameter of 100 to 140 nm by Gaussian analysis and were stored at 4.degree. C. until use.

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L2: Entry 7 of 27

File: USPT

Sep 7, 1999

US-PAT-NO: 5948441

DOCUMENT-IDENTIFIER: US 5948441 A

TITLE: Method for size separation of particles

DATE-ISSUED: September 7, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: [424/489](#); [264/4.1](#), [264/4.3](#), [424/450](#), [424/502](#), [428/402.2](#), [436/829](#),
[514/78](#)

CLAIMS:

We claim:

1. A method of producing nonliposomal lipid particles of a homogeneous, defined size distribution from a mixture of lipid particles of heterogeneous size comprising the steps of:

(a) subjecting the mixture to tangential flow filtration with a first filter of a first pore size;

(b) subjecting the filtrate from step (a) to tangential flow filtration with a second filter of a second, smaller pore size; and

(c) collecting the retentate from step (b),

wherein the first pore size defines the upper limit of the size distribution of the liposomes or lipid particles, the first pore size is between about 10 and about 0.2 microns, the second pore size defines the lower limit of size distribution of the particles and the second pore size is between about 2000 molecular weight and about 2 microns.

2. The method of claim 1 wherein the lipid particles additionally comprise a bioactive agent.

3. The method of claim 2 wherein the bioactive agent comprises a polyene antifungal agent.

4. The method of claim 3 wherein the polyene antifungal agent comprises amphotericin B and wherein the lipid particles comprise a lipid which comprises dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol.
5. The method of claim 1 wherein the first pore size is about 5 um.
6. The method of claim 1 wherein the second pore size is about 1.0 um.
7. The method of claim 1 further comprising the step of milling the lipid particles to reduce their size prior to their application to a tangential flow filter.
8. A population of lipid particles with a homogeneous defined size distribution prepared by tangential flow filtration in accordance with the method of claim 1.
9. The population of claim 8 wherein the lipid particles comprise a lipid and a bioactive agent.
10. A method of producing nonliposomal lipid particles of a size having sizes below a defined size cutoff comprising the steps of:
 - (a) homogenizing the particles:
 - (b) subjecting the particles to tangential flow filtration with a first filter of a pore size which excludes particles above the defined cutoff;
 - (c) collecting the filtrate from step (b) and,
 - (d) subjecting the collected filtrate from step (c) to tangential flow filtration with a second filter of smaller pore size.
11. A method for preparing a liposome or lipid particle comprising the step of contacting a solution containing lipid to a first side of a filter in tangential flow filtration apparatus while infusing or injecting an aqueous solution to a second side of the filter in the tangential flow filtration apparatus.
12. The method of claim 11 wherein the tangential flow filtration system employed is a dynamic rotary filtration or a hollow fiber filtration.

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